Rule 1.126

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114. ~~112.~~ The vaccine of claim 55 formulated for mucosal delivery, wherein the vaccine is administered orally or intranasally.

**REMARKS**

Timely consideration of the application is respectfully requested in light of the amendment and the following remarks. Claims 55-112 are currently pending. Support for the newly added claim can be found at the paragraph bridging pages 74-75. Thus, no prohibited new matter has been added by the amendment.

## II. Conclusion

Should the Examiner feel that there are any issues outstanding after consideration of this Supplemental Amendment, the Examiner is invited to contact the Applicants' undersigned representative to expedite prosecution.

In the unlikely event that the transmittal letter submitted herewith is separated from this document, and **except** for issue fees payable under 37 C.F.R. §1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application, including fees due under 37 C.F.R. §§ 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account No. 50-0310. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 C.F.R. § 1.136(a)(3).

Respectfully Submitted,

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Date: July 30, 2001

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# L-selectin-mediated lymphocyte rolling on MAdCAM-1

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**THE L-selectin, a cell surface C-type lectin, directs lymphocyte traffic to lymph nodes[1], and contributes to lymphocyte homing to Peyer's patches[2,3] and to leukocyte interactions with inflamed venules[4,5]. Here we report that the mucosal vascular addressin MAdCAM-1, a mucosal endothelial adhesion molecule with immunoglobulin- and mucin-like domains[6], is a facultative ligand for L-selectin. MAdCAM-1 isolated from mesenteric lymph nodes, but not from cultured endothelioma cells, bears *N*-glycanase-resistant sialic acid-containing carbohydrate which supports adhesion of L-selectin-transfected lymphoid cells under shear. Interacting lymphoid cells display a 'rolling' behaviour similar to the selectin-dependent rolling of neutrophils observed in inflamed venules. MAdCAM-1 is also a ligand for the lymphocyte integrin homing receptor for Peyer's patches, $\alpha 4\beta 7$ (ref. 7), and may be uniquely adapted to support both selectin-mediated lymphocyte rolling and integrin-mediated adhesion and arrest *in vivo*.**

High endothelial venule (HEV) ligands for the lymphocyte L-selectin homing receptor display an epitope defined by the peripheral lymph node addressin-specific monoclonal antibody MECA-79: MECA-79 preferentially reacts with L-selectin-binding HEV, inhibits L-selectin-dependent lymphocyte binding to HEV *in vitro* and *in vivo*, and immunoprecipitates HEV molecules that bind lymphocytes and L-selectin transfectants[8,9] or L-selectin-immunoglobulin chimaeric proteins[10,11]. MECA-79 reactivity and L-selectin binding may be conferred by post-translational carbohydrate modifications that are HEV-specific but that can decorate multiple glycoprotein species[8]. When studying MAdCAM-1 isolated from mouse Peyer's patches, we found that it is precipitated not only by anti-MAdCAM-1 antibody MECA-367, but also by MECA-79 (Fig. 1*a*). Serial immunoprecipitation with MECA-79 was unable to preclear MAdCAM-1 from Peyer's patches completely, suggesting that the MECA-79 epitope decorates only a subset of MAdCAM-1 (Fig. 1*b*). MECA-79 also binds in western blots to MAdCAM-1 isolated from mesenteric lymph nodes (MLN) (Fig. 1*c*), confirming that MAdCAM-1 can be directly modified by the MECA-79 epitope.

We also assessed the presence of MAdCAM-1 in MECA-79-isolated addressin from peripheral lymph nodes (PLN) of young mice, whose PLN still retain some of the MAdCAM-1 expression that characterizes PLN-HEV in the early postnatal period. As previously described for human PLN addressin[8], a MECA-79 affinity column binds multiple iodinatible glycoprotein species (Fig. 2). Immunoprecipitated species, which are also recognized prominently by MECA-79 in western blots (not shown), are indicated by arrows (Fig. 2, lane 1). Isolated species include MAdCAM-1, as shown by immunoprecipitation with MECA-367 (Fig. 2, lane 4). The 60K band in MECA-79 immunoprecipitates (double arrows, lane 1) is precleared by anti-MAdCAM-1 antibodies (not shown). These data indicate that MAdCAM-1 can accept the MECA-79 epitope when expressed by HEV. In contrast, MAdCAM-1 isolated from tumour necrosis factor (TNF)-$\alpha$-stimulated cultured endothelioma cells, or from lymphoid cells transfected with MAdCAM-1 complementary DNA, does not bear the MECA-79 epitope (results not shown, and see below).

The cDNA sequence of MAdCAM-1 predicts a prominent mucin-like domain, a potential site of *O*-glycosylation[6], and three potential *N*-glycosylation sites. Treatment of immunoisolated, iodinated MLN MAdCAM-1 with peptide *N*-glycosidase F (to detect *N*-linked sites) did not alter the relative molecular mass, indicating either that the *N*-glycosylation sites are not used, or that any *N*-linked glycans present are resistant to this enzyme (Fig. 3, lane 2). Neuraminidase treatment increased the migration of MAdCAM-1 in SDS–PAGE (Fig. 3, lane 4). Endo-$\alpha$-*N*-acetyl-D-galactosaminidase (used to detect *O*-linked sites) had no effect on native (not shown) or neuraminidase-treated MAdCAM-1 (Fig. 3, lane 5). As the activity of this enzyme is largely restricted to a subset of simpler *O*-linked carbohydrates[12], this result is consistent with the presence of complex or modified, sialic acid-bearing *O*-linked carbohydrate. Demonstrable cleavage of the control protein CD44 with all of the enzymes used confirms their activities (Fig. 3, lanes 8–14). It is likely that *O*-linked carbohydrate associated with the mucin-like extracellular domain of MAdCAM-1 represents the site of modification with the MECA-79 epitope.

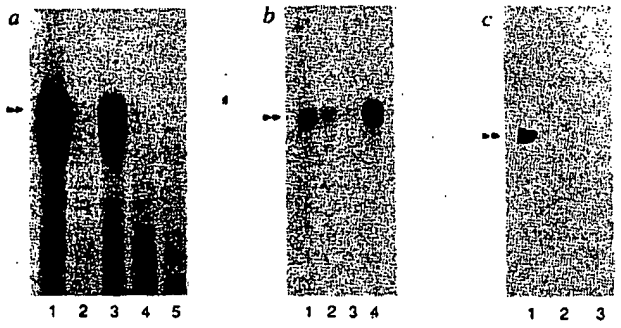


FIG. 1 MECA-79 recognizes MAdCAM-1. *a*, MECA-79 immunoprecipitates purified MAdCAM-1. Immunoisolated MAdCAM-1 from Peyer's patches (PP) was iodinated and precipitated with MECA-367 (lane 1), a rat IgG2a control antibody (lane 2), MECA-79 (lane 3), or rat IgM control antibodies (lanes 4,5). *b*, Only a subset of MAdCAM-1 can be precleared by MECA-79. Immunoisolated, iodinated MAd from PP was precipitated with MECA-79 (lanes 1–3) or MECA-367 (lane 4), before (lane 1) or after preclearing with MECA-79 once (lane 2), twice (lane 3), or three (lane 4) times. The double arrows indicate the $M_r$ of MAdCAM-1 at 58–66K. *c*, MECA-79 reacts with immunoisolated MAdCAM-1 by western blot. Immunoisolated MECA-367 binding material from MLN was applied to an SDS–PAGE gel under non-reducing conditions. After transfer to nitrocellulose, the blot was probed with MECA-79 (lane 1), or two control rat IgM antibodies (lanes 2 and 3). The double arrows indicate the $M_r$ of MAdCAM-1 which is 54–62K under non-reducing conditions[24].

METHODS. *a*, *b*, MAdCAM-1 was prepared from PP stromal lysates of 20 BALB/c mice by immmunoaffinity chromatography with MECA-367 as described[7,24]. A sample from the peak column fraction was then iodinated with 1.8 mCi $Na^{125}I$ (specific activity 15 mCi $\mu g^{-1}$) as described[25]. The radiolabelled antigen was immunoprecipitated with the appropriate antibody-coupled Sepharose beads as indicated. For this, samples were diluted with NP-40 wash buffer[7] containing 0.1% haemaglobin (Hb/WB) and the appropriate antibody-coupled beads added. After 30–60 min room temperature incubation, the beads were washed by centrifugation of the beads over 10% and 20% sucrose cushions in Hb/WB, followed by Hb/WB again and then 10 mM Tris–HCl, pH 6.8 containing 0.1% SDS. Material specifically adsorbing to the beads was released by incubation in $\beta$-mercaptoethanol-containing solubilization buffer for analysis by SDS–PAGE and subsequent autoradiography. *c*, MLN-MAdCAM-1 was immunoisolated from MLN stromal lysates of 300 mice as previously described[7,21]. Samples of MLN-MAdCAM-1 were run on SDS–PAGE gels, transferred to nitrocellulose paper (NC, BioRad), and western blots done as described[24]. Primary antibodies included 100 $\mu g\ ml^{-1}$ purified MECA-79, or control rat IgM antibodies in 10% horse serum (HS) in RPMI medium. The secondary antibody used was alkaline phosphatase-conjugated rabbit anti-rat IgM (Zymed) in 10% HS in RPMI at 1:200 dilution.

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The presence of the MECA-79 epitope suggested that MAdCAM-1 might be decorated by L-selectin-binding carbohydrates. To test this possibility, glass capillary tubes coated with immunoisolated MLN MAdCAM-1 were infused with mouse L1-2 pre-B cells transfected with human L-selectin. L1-2 cells lack the $\alpha 4\beta 7$ integrin receptor for MAdCAM-1, thus allowing assessment of L-selectin-mediated adhesion in the absence of complicating integrin interactions. As seen in Fig. 4*A*, L1-2 cells transfected with L-selectin (*a*), but not vector (*b*) interact with MLN MAdCAM-1. The interaction is loose, resulting in 'rolling' of transfectants, a behaviour characteristic of selectin-mediated adhesion under flow[5,13]. Rolling is blocked by anti-L-selectin monoclonal antibody Dreg-56 (Fig. 4*A*, *c*) and by preincubation of the coated MAdCAM-1 with neuraminidase (Fig. 4*B*, *a*). Importantly, the L-selectin transfectants displayed no rolling on the MAdCAM-1 isolated from MAdCAM-1-transfected lymphoid cells (not shown) or from TNF-$\alpha$-stimulated cultured bEnd.3 endothelioma cells (Fig. 4*B*, *b*), although the bEnd.3 MAdCAM-1 was as potent a substrate or slightly better than MLN MAdCAM-1 for $\alpha 4\beta 7$-restricted interactions as assessed by adhesion of TK-1 cells, an $\alpha 4\beta 7$-positive mucosal HEV-binding cell line[7,14] (Fig. 4 legend). We conclude that MAdCAM-1 isolated from lymph nodes supports neuraminidase-sensitive L-selectin-dependent lymphocyte rolling.

In conclusion, MAdCAM-1 expressed by HEV can be modified by carbohydrates comprising the binding sites for the lymphocyte homing receptor L-selectin. Modified MAdCAM-1 represents the first identified high endothelial cell molecule shown to support L-selectin-dependent lymphocyte rolling. Like the independently identified HEV-associated L-selectin ligands GlyCAM-1 (a secreted molecule)[15,16] and CD34[11,17], MAdCAM-1 features a prominent mucin-like domain that may contribute to efficient display of carbohydrate determinants through enhanced valency. MAdCAM-1 is well positioned to contribute to L-selectin-mediated lymphocyte interactions in MLN and Peyer's patches, whose HEV coordinately express high levels of MAdCAM-1 with high (MLN) or low but significant (Peyer's patches) MECA-79 and L-selectin-immunoglobulin reactivity[2,3,18]. It is unlikely to play a significant role as an L-selectin ligand in adult non-mucosal lymph nodes, in which its expression is downregulated, or in lamina propria venules which display MAdCAM-1 but no detectable MECA-79 or L-selectin immunoglobulin binding activity. MAdCAM-1 also binds lymphocyte $\alpha 4\beta 7$ integrin[7], an adhesion pathway that confers specificity to PP homing[19,20] and dominates MAdCAM-1 binding in non-flow *in vitro* assays[7,21]. MAdCAM-1 is thus a multifunctional molecule capable of serving as a vascular receptor for both the leukocyte selectin and for leukocyte integrins. In this regard, MAdCAM-1 represents an elegant example of the expression of multiple adhesive motifs within a single molecule, motifs that may at least cooperate and potentially synergize in support of lymphocyte–HEV interactions. In the context of recent models of lymphocyte-endothelial cell recognition as a multistep process[22,23], MAdCAM-1 expressed in certain *in vivo* sites may in fact be able to participate in both selectin-mediated primary

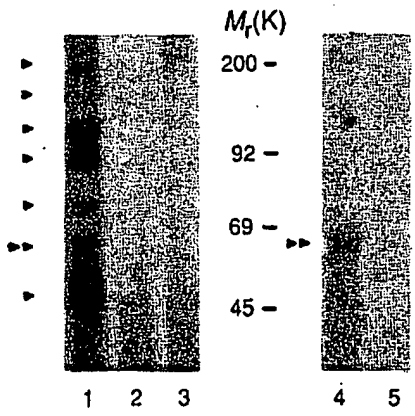


FIG. 2 Anti MAdCAM-1 antibody MECA-367 immunoprecipitates a 60K lymph node antigen that binds to MECA-79. Immunoisolated MECA-79 antigen was iodinated, reprecipitated with MECA-79 (lane 1), control rat IgM antibodies (lanes 2 and 3), MECA-367 (lane 4) or control rat IgG2a antibody (lane 5), and electrophoresed on an SDS–PAGE gel under reducing conditions. The seven major species which are also reactive with MECA-79 in western blots (under both reducing and non-reducing conditions, not shown) are indicated by arrows at 200, 170, 115, 90, 75, ~60 and 50K. The double arrows indicate a 60K species which comigrates with MAdCAM-1. PLN of young BALB/c mice (7 weeks) were used for this experiment because they represent a more concentrated source of MECA-79 antigen, yet still retain some of the MAdCAM-1 expression that characterizes PLN-HEV in the early postnatal period[26]. METHODS. MECA-79 antigens were isolated from PLN stromal lysates of 20 seven-week-old BALB/c mice by immunoaffinity chromatography generally as described previously and above[8,24]. A sample from the peak column fraction was then iodinated and immunoprecipitated as described in Fig. 1.

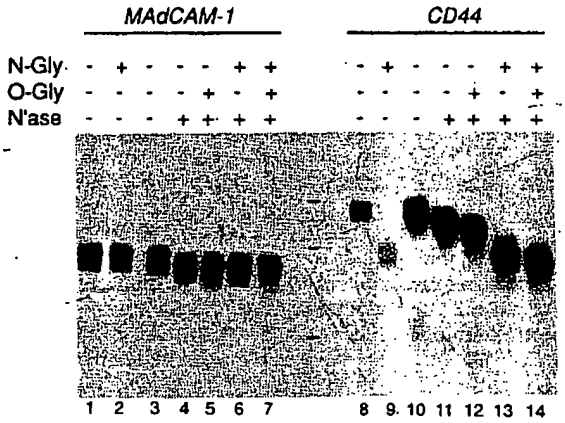


FIG. 3 MAdCAM-1 immunoisolated from MLN contains *N*- and *O*-glycosidase-insensitive sialic acid containing carbohydrate. MAdCAM-1 migration on gels is affected by treatment with neuraminidase but not peptide-*N*-glycosidase F (*N*-Gly) or endo-$\alpha$-*N*-acetyl-D-galactosaminidase (O-Gly) whereas the control glycoprotein CD44, which contains significant amounts of both *N*- and *O*-Gly-sensitive carbohydrate, is affected by all of the enzymes used. Immunoisolated MLN MAdCAM-1, or a control protein, CD44, isolated from human foreskin fibroblast cells[28] was radioiodinated, enzymatically digested, then applied to 8% SDS–PAGE gels under reducing conditions. Autoradiograms prepared from the resulting gels show MAdCAM-1 (lanes 1–7) or CD44 (lanes 8–14) treated with control buffer (lanes 1 and 3; 8 and 10), *N*-Gly (lanes 2 and 9), *Vibrio cholera* neuraminidase (N'ase, lanes 4 and 11), neuraminidase plus *O*-Gly (lanes 5 and 12), neuraminidase plus *N*-Gly (lanes 6 and 13), or all three enzymes (lanes 7 and 14). In experiments not shown, the effect of the neuraminidase treatment on MAdCAM-1 could be blocked by including 10 mM sialyllactose or 5 mM EDTA in the reaction buffer, demonstrating the specificity of the enzyme.
METHODS. The MAdCAM-1 used was isolated and iodinated from MLN as described in Fig. 1. Samples were immunoprecipitated with either MECA-367- or anti-CD44-coupled Sepharose beads[25] and then were boiled in 0.15% SDS, 40 mM $\beta$-mercaptoethanol before addition of NP-40 for a final concentration of 1.5%. Neuraminidase treatment was done in buffer containing 50 mM $NaH_2PO_4$, pH 6.0, 5 mM $CaCl_2$ with 2.5 mU *Vibrio cholera* neuraminidase (Boehringer Mannheim) for 6 h at 37°C. Treatment with 0.5 mU endo-$\alpha$-*N*-acetyl-galactosaminidase (O-Gly; Boehringer Mannheim) was done in the same buffer for 16 h at 37°C. For peptide-*N*-glycosidase F (*N*-Gly; Genzyme) digestion, the pH was adjusted to 8.6 before the addition of 0.1 U and incubation for 7–17 h at 37°C. An equal volume of SDS solubilization buffer containing $\beta$-mercaptoethanol was added to each sample before SDS–PAGE analysis and subsequent autoradiography.

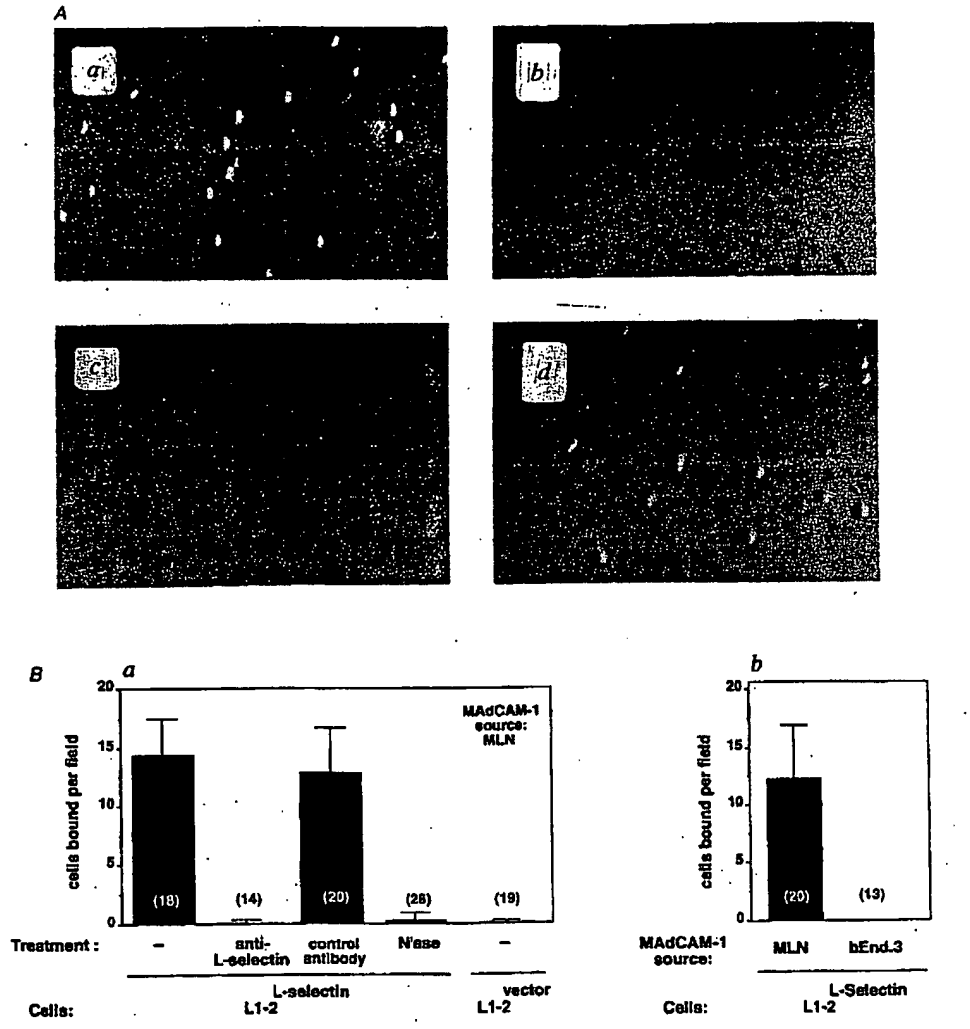


FIG. 4 MAdCAM-1 isolated from mouse MLN supports L-selectin-mediated lymphocyte rolling under shear. *A*, L1–2 cells transfected with L-selectin (L1–$2^{L\text{-selectin}}$) were infused into glass capillary tubes coated with MAdCAM-1 derived from mouse MLN. L1–$2^{L\text{-selectin}}$ cells (*a*) but not control vector transfectants, L1–$2^{vector}$ (*b*) interact with regions of the tube coated with MLN MAdCAM-1. Interaction of L1–$2^{L\text{-selectin}}$ cells with MLN MAdCAM-1 is blocked in the presence of the anti-L-selectin antibody Dreg-56 (ref. 27) (*c*), but not isotype control antibody (*d*). *B*, L-selectin-mediated lymphocyte interactions with MAdCAM-1-coated glass tubes under shear stress. *a*, L-selectin transfectant rolling on MLN MAdCAM-1 is blocked by treatment with the anti-L-selectin antibody Dreg-56 and also by treatment of MLN MAdCAM-1 with neuraminidase. *b*, L-selectin transfectants interact with MAdCAM-1 isolated from MLN but not from TNF-$\alpha$-stimulated bEnd.3 endothelioma cells. L1–$2^{L\text{-selectin}}$ or L1–$2^{vector}$ transfectants were infused into glass capillary tubes coated with MAdCAM-1. As described, each experiment was recorded on video tape. After a 1-min period to establish equilibrium, the number of cells interacting with the tube in individual frames 4–5 s apart, over the next 90 s, were counted and the average number of cells and the standard deviation obtained is reported. For *b*, both tubes were initiated simultaneously, thus the results obtained for each data point are derived from two 30-s periods of observation over a total of 2 min. The data shown are from individual experiments but were repeated 3–5 times with similar results. The number of frames averaged for each data point is shown in parentheses.

METHODS. MAdCAM-1 was immunoisolated either from MLN stromal lysates from 300 ICR mice or 10 T-175 flasks of the hIMAd-4 subline of the bEnd.3 endothelioma cell line, grown and stimulated for 18 h with 50 U $ml^{-1}$ TNF-$\alpha$ as described[6]. Isolation followed previously described methods[7,8,21]. The bEnd.3 and MLN MAdCAM-1 preparations used were normalized by immunoreactivity with MECA-367 by ELISA. In conventional, non-flow adhesion assays as described by Berlin *et al.*[7], the bEnd.3 MAdCAM-1 was slightly more potent than MLN MAdCAM-1 in mediating $\alpha4\beta7$-restricted adhesion as assessed by the $\alpha4\beta7^{+}$L-selectin$^{-}$ mucosal HEV binding cell line TK-1[7] (the number of cells binding to MLN MAdCAM-1 versus bEnd.3 MAdCAM-1 was $32.4 \pm 13.7$ s.e.m., $n = 7$ fields, versus $40.1 \pm 12$ s.e.m., $n = 7$ fields). To examine the ability of MAdCAM-1 to support L-selectin-dependent adhesion under conditions of shear, samples of immunoisolated material were absorbed onto the inside surface of one end of 4-inch-long glass capillary tubes (N51A glass, 1.415 mm internal diameter; Drummond Scientific, Broomall, PA) by dilution of the material in calcium and magnesium-containing Hank's Balanced Salt Solution (HBSS; 1:15). After overnight incubation, the tubes were washed and blocked with 5% normal bovine serum, 20 mM HEPES, pH 7.3 in HBSS for 30 min at room temperature. The development and growth of mouse L1–2 pre-B cell lines transfected with human L-selectin cDNA (L1–$2^{L\text{-selectin}}$) or vector cDNA (L1–$2^{vector}$) have been previously described[8,28]. Transfectants were grown to $2 \times 10^6$ cells per ml and then infused at room temperature in the capillary tube at a flow rate of 2.6 ml $min^{-1}$ with a syringe pump (Sage Instruments, Model 351) fitted with a 12-ml syringe. The coated portion of the tube was ~4 cm from the tube connected to the syringe pump to allow the establishment of laminar flow. This flow rate in this diameter tube corresponds to a wall shear rate of 71 $s^{-1}$ and, assuming a viscosity of the serum-containing medium at room temperature of ~0.015 poise, this corresponds to a wall shear stress of 1.1 dynes $cm^{-1}$ (refs 13, 29) and allows ~4 min continuous observation per sample. The conditions used were not intended to represent physiological conditions which must include among many other parameters, the rheological and anatomical considerations as in ref. 30, which describes the path of lymphocytes through HEV as tortuous and 'pinball-like'. This method does allow the study of adhesive interactions under conditions of defined flow. In some experiments, monoclonal antibodies were added (20 μg $ml^{-1}$) to the cells 5 min before the assay. The interaction of cells with the tube surface was viewed with a Nikon inverted microscope and the entire experiment recorded with a video monitor. At the flow rate indicated, cells interacting with the tube surface are easily distinguished from non-interacting cells. To quantify the interaction, after an initial 60-s period in which equilibrium was established, three 150 power fields ($400 \times 500\ \mu m^2$) were observed for 30 s each (for a total observation period of 90 s. On review of the experiments, the video tape was paused every 4–7 s and the number of cells interacting with the tube wall could be counted. In some experiments, the addressin-coated tubes were treated with neuraminidase (*Vibrio cholera* neuraminidase, Calbiochem) 5 mU $ml^{-1}$ in HBSS for 10 min before washing with NBS-containing medium and the addition of cells. All interacting cells were actively rolling, although a few cells (*A*,*a*) would occasionally pause for 2–3 s before moving onward. The mean rolling velocity of L-selectin transfectants on MLN-derived MAdCAM-1 was determined in one experiment and shown to be $141 \pm 68\ \mu m\ s^{-1}$ s.d. ($n = 20$ cells).

adhesion and rolling under conditions of physiological shear, and in integrin-mediated binding interactions as well, including activation-dependent sticking and arrest. It will be important to assay directly, through *in situ* analysis, the involvement of MAdCAM-1 and of other putative HEV ligands in L-selectin-dependent lymphocyte–HEV interactions *in vivo*. □

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# A first-generation physical map of the human genome

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**Sets of ordered overlapping cloned genomic DNA fragments that span each of the human chromosomes are urgently needed for identification of human disease genes. Such a physical map also provides unique material to study the structure and function of the genome. We have therefore exhaustively analysed the CEPH yeast artificial chromosome (YAC) library, which contains 33,000 clones, whose insert size was individually determined. These YACs have an average length of 0.9 megabases and cover the equivalent of 10 haploid genomes. Several mapping techniques were combined to provide multiple sources of structural information for most of these clones. Finally, the library was screened with more than 2,000 genetic markers quasiuniformly distributed over 90% of the genome. These results should allow the scientific community to construct detailed maps of all human chromosomes. Moreover, we propose a data analysis strategy that produces a first-generation integrated map covering most of the human genome.**

Several different methods have been used to construct genomic physical maps. All these techniques establish overlaps between clones that allow the reconstitution of the original genomic order, but each method has its limitations. We therefore decided to combine four major techniques to generate structural and positional data from a large number of YAC clones.

The restriction fragment pattern of individual clones has been used successfully in the past to establish maps of *Escherichia coli*[1], yeast[2] and *Caenorhabditis elegans*[3]. We produced similar fingerprints for all 33,000 YACs of our library by detection of medium-repeat sequences (THE and L1) containing fragments generated by three enzymes, as described[4].

Single-copy landmark screening also established overlaps[5], and limited application of this approach has already provided maps for two of the smallest human chromosomes[6,7]. If these landmarks are polymorphic sequence-tagged sites (STSs)[7], they can be genetically ordered using meiotic recombination and assigned to specific chromosomes. We used 2,100 polymorphic genetically mapped STSs (ref. 9; and J. W. *et al.*, submitted) to screen partially or totally the same 33,000 YACs. We have obtained an average of 5.52 YACs that are positive for a selected subset of 1,068 STSs, and 2.05 clones for another 714. This allowed us to determine the STS content of 6,580 clones (20% of the total library).

We also used another approach for rapid and extensive single-copy landmark screening based on the use of YACs as hybridization probes. We produced specific sequences for each clone by polymerase chain reaction (PCR) amplification between the ubiquitous Alu repeats[10–12]. We derived PCR products for a subset of 25,000 YACs with a mean size of 1.1 megabases (Mb), representing 9 genome equivalents. After rational pooling[7], these products were spotted at high density onto membranes together with inter-Alu PCR products from a monochromosomal somatic cell hybrid mapping panel[13]. These membranes were successfully hybridized to 5,332 inter-Alu PCR probes derived by amplification of individual YACs representing at least 2 genome equivalents. We also preferentially used polymorphic STSs containing YACs (2100), together with clones from chromosome-specific sublibraries derived for each human chromosome[12]. On average, for each YAC probe, 10 YACs were detected. In this way we established homology relations among clones in a set of 20,750 YACs, that included both YAC probes and their targets. This number corresponds to 83% of the library screened. We were able to assign simultaneously 4,373 YAC probes out of 5,332 YAC probes, to human chromosomes and deduce possible chromosomal locations for more than 15,000 target YACs.

Finally, about 500 YACs containing genetically mapped polymorphic STSs (one every 7.4 centimorgans) were positioned on metaphase chromosomes using FISH[14]. This allowed the integration of genetic, physical and cytogenic maps. All these data, together with clone sizes, are available to the scientific community by anonymous FTP (File Transfer Protocol) from ceph-genethon-map.genethon.fr or by electronic mail requests to ceph-genethon-map@ genethon. genethon.fr.. Specifically, files with STS content of clones, results of Alu PCR hybridizations, L1 and the fingerprint band sizes of individual YACs and relationships between clones, deduced from fingerprint analysis, together with sizes of clones and their FISH localization will be available by FTP. Additional information on the results of screenings will be initially provided through electronic mail contacts. Our library has already been freely distributed since 1992[15].

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## The human peripheral lymph node vascular addressin is a ligand for LECAM-1, the peripheral lymph node homing receptor.

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The trafficking of lymphocytes from the blood and into lymphoid organs is controlled by tissue-selective lymphocyte interactions with specialized endothelial cells lining post capillary venules, in particular the high endothelial venules (HEV) found in lymphoid tissues and sites of chronic inflammation. Lymphocyte interactions with HEV are mediated in part by lymphocyte homing receptors and tissue-specific HEV determinants, the vascular addressins. A peripheral lymph node addressin (PNAd) has been detected immunohistologically in mouse and man by monoclonal antibody MECA-79, which inhibits lymphocyte homing to lymph nodes and lymphocyte binding to lymph node and tonsillar HEV. The human MECA-79 antigen, PNAd, is molecularly distinct from the 65-kD mucosal vascular addressin. The most abundant iodinated species by SDS-PAGE is 105 kD. When affinity isolated and immobilized on glass slides, MECA-79 immunoisolated material binds human and mouse lymphocytes avidly in a calcium dependent manner. Binding is blocked by mAb MECA-79, by antibodies against mouse or human LECAM-1 (the peripheral lymph node homing receptor, the MEL-14 antigen, LAM-1), and by treatment of PNAd with neuraminidase. Expression of LECAM-1 cDNA confers PNAd binding ability on a transfected B cell line. We conclude that LECAM-1 mediates lymphocyte binding to PNAd, an interaction that involves the lectin activity of LECAM-1 and carbohydrate determinants on the addressin.

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